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Identification and functional
characterization of adhesins
involved in
attachment of methanogens to
rumen protozoa

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Abstract

Symbiotic interactions are frequently observed amongst members of the complex microbial community inhabiting the fermentative forestomach (rumen) of ruminant animals. In this ecosystem, hydrogen (H₂)-using methanogens can be found as ecto- and endo-symbionts of H₂-producing protozoa, and this interaction contributes to ruminant methane emissions. Rumen symbionts must have the ability to attach to protozoal hosts, presumably *via* protozoa-binding cell surface proteins, however the identity and specificity of these proteins are not known.

A protein of the methanogenic archaeon *Methanobrevibacter ruminantium* M1 that binds to rumen protozoa was identified using phage display technology. A large shot-gun phage display library was constructed from M1 DNA, and affinity screened by biopanning using rumen protozoa as bait. After two rounds of biopanning, a recombinant clone encoding part of a previously annotated putative adhesin, Mru_1499, was identified as a protozoa-binding protein. The protozoal binding region of the affinity selected protein was mapped, and a “reverse panning” procedure was developed to identify protozoal species that bind to the affinity selected protein.

Next, the protozoa-associated methanogen and bacterial communities were characterized, and several taxa of archaea and bacteria were found to be over-represented in the protozoa-associated community relative to their abundance in the rumen contents. Adhesins from this protozoa-associated community were identified by affinity screening of a community-scale phage display library using rumen protozoa as bait, combined with high-throughput single molecule amplicon sequencing. The comparison between pre- and post-panning sequence datasets showed seven highly enriched candidate adhesin-encoding ORFs after affinity-panning of the library on protozoa as bait.

In conclusion, several adhesins mediating interactions between methanogenic archaea, bacteria and protozoa were identified using phage display at both single-organism and metagenome scales. Further assays are required to verify the function of these candidate adhesins as “molecular bridges” in interactions involving rumen protozoa. This is the first report for characterization of the protozoa-associated symbiont community by next generation sequencing of the 16S rRNA gene.

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Abbreviations

ALP	Adhesin-like protein
BSA	Bovine serum albumin
cfu	Colony forming units
DGGE	Denaturing gradient gel electrophoresis
EDS	Energy dispersive X-ray spectroscopy
FISH	Fluorescence <i>in situ</i> hybridization
GAPDH	Glycerladehyde-3-phosphate dehydrogenase
GlcNac	<i>N</i> -acetylglucosamine
Ig-like	Immunoglobulin-like
IQR	Interquartile range
m.o.i.	Multiplicity of infection
nr	Non-redundant
OD	Optical density
ORF	Open reading frame
OTU	Operational taxonomic unit
PAM1	Shot-gun phage display primary library created from metagenomic DNA
PAM2	Shot-gun phage display primary library after affinity selection against c-myc
PAM_FINAL	PAM2 library after two rounds of biopanning against protozoal bait, followed by affinity selection against c-myc
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
pfu	Plaque forming units
PMB	Pseudomurein binding
pMru_1499 ^A	Phagemid vector encoding Mru_1499 ^A
PP	Phagemid particle
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
TBS-T	Tris buffered saline containing Tween 20